THE NITROGENOUS METABOLISM OF BACILLUS COLI

STUDIES IN BACTERIAL METABOLISM. LXII

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Bacillus coli is normally the most abundant organism of the lower intestinal contents in adolescence and adult life. Observations are not wanting which would suggest that it is equally commonly found in the feces of domestic animals. Even in severe infections of the intestinal tract by exogenous bacteria, such as typhoid or dysentery bacilli, the colon bacillus persists in variable but relatively considerable numbers, and protracted starvation fails to eliminate the microbe.[1] It appears to possess characteristics which meet those of the intestinal environment without apparent discomfort to the host.

Chemically and culturally, B. coli is distinctly more active than those exogenous intestinal invaders which incite specific disease, such as B. typhosus and members of the dysentery bacillus group. The colon bacillus is less active chemically than the cholera group, however. In general, it may be stated that intense chemical and cultural activity is incompatible with etiologic participation in progressively pathogenic disease. Cholera is apparently a striking exception to this statement, however.

Previous studies of the nitrogenous metabolism of certain members of the pathogenic intestinal group of bacilli[2] have shown that the changes in the nitrogenous constituents of ordinary mediums (containing no utilizable carbohydrate) which are utilized for energy are chiefly centered in that portion of the nitrogen spectrum designated, for purposes of discussion, "polypeptid" nitrogen.[3] This fraction consists of the polypeptids, creatin and creatinin, purin and pyrimidin bases chiefly. The diminution of this polypeptid fraction as growth proceeds, aside from a small increase in free ammonia indicating the utilization of some amino acid for the requisite energy of the microbes, is closely

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[1] Kendall: Observations on the Bacterial Intestinal Flora of a Starving Man. Publication 203, Carnegie Institute, Washington, 1915, p. 232.

[2] Studies LVII-LXI, inclusive, Jour. Infect. Dis., 1922, 30, pp. 211-238.

[3] Kendall: Study LVII, ibid., 1922, 30, p. 211.

paralleled by a corresponding increase in the protein-nitrogen fraction of the nitrogen content. The most direct explanation for this apparent transformation of nonprotein nitrogen is that an actual production of bacterial protein has taken place. In other words, this is a measure of the growth of bacteria in the medium.

It should be stated that centrifugalization of such a culture will throw out an amount of nitrogen almost exactly equal to the increase of bacterial nitrogen as growth proceeds. A microscopic examination of the sediment will reveal the fact that it is essentially made up of the bodies of bacteria. Within the limits of precision of available methods, therefore, pathogenic bacteria of the typhoid-dysentery-paratyphoid group appear to build their bodies from the "polypeptid" fraction of ordinary nutrient gelatin mediums, rather than from the gelatin protein which is also present. The absence of certain aromatic amino acids from gelatin, such as tyrosin, may be of some significance in this connection, although the evidence is on the whole against it.

If utilizable carbohydrate is present in the gelatin medium, the requisite energy of the microbes is obtained from it; the increment in the protein nitrogen occurs without appreciable change in the free ammonia. If, on the contrary, protein and protein derivatives alone are present, the protein fraction increases with a concomitant augmentation in free ammonia, suggesting the utilization of a certain amount of nitrogen-containing substance for energy.

As B. coli is more reactive than the pathogenic intestinal bacteria referred to in the foregoing, it is a matter of some theoretical importance to determine the effects of its growth on nitrogenous constituents of cultural mediums. Nutrient gelatin, both with and without the addition of glucose, was selected because the protein fraction is much greater in this enriched medium than would be the case in the peptone mediums usually employed for cultural observations.

The same series of observations as those reported in previous studies were made in cultures of B. coli.[2] In addition, an effort was made to determine whether a soluble proteolytic enzyme was present, which might cause cleavage of some of the gelatin or peptone protein. The method used to detect such an enzyme has been described previously.[4] It consists essentially in adding 5 c c of a growing culture of the organism at stated intervals to 95 c c of a 5% solution of gelatin in water to which is added 0.5% phenol. The phenol prevents the

[4] Kendall and Walker: Jour. Infect. Dis., 1915, 17, p. 442.

growth of the bacteria, and experience has shown that the soluble enzyme of organisms, such as B. proteus, is not prevented from acting in such a medium. It is necessary to make careful controls each time a culture is examined for enzyme activity. One control is examined immediately after the culture is added. Incubation of the culture—carbol gelatin mixture is practiced 3 days to afford time for the enzyme to act.

TABLE 1

BACILLUS COLI

Mg. per 100 C c	Control	Day	Plain Broth	Glucose Broth
Total nitrogen	1.064	1	1.064	1.064
Protein nitrogen	0.762		0.773	0.773
Nonprotein nitrogen	0.302		0.291	0.291
Polypeptid nitrogen	0.213		0.202	0.208
Amino nitrogen	0.032		0.022	0.032
Ammonia nitrogen	0.057		0.067	0.056
Reaction	+0.60		+0.20	+3.80
PH	7.0		.....	.....
Total nitrogen	1.064	3	1.064	1.064
Protein nitrogen	0.762		0.840	0.840
Nonprotein nitrogen	0.302		0.224	0.244
Polypeptid nitrogen	0.213		0.137	0.144
Amino nitrogen	0.032		0.014	0.030
Ammonia nitrogen	0.057		0.073	0.050
Reaction	+0.60		−1.40	+5.50
PH	7.0		7.8	5.0
Total nitrogen	1.064	6	1.064	1.064
Protein nitrogen	0.762		0.863	0.885
Nonprotein nitrogen	0.302		0.201	0.179
Polypeptid nitrogen	0.213		0.092	0.092
Amino nitrogen	0.032		0.028	0.037
Ammonia nitrogen	0.057		0.081	0.050
Reaction	+0.60		−1.70	+5.80
PH	7.0		8.1	4.9
Total nitrogen	1.064	10	1.064	1.064
Protein nitrogen	0.762		0.840	0.852
Nonprotein nitrogen	0.302		0.224	0.212
Polypeptid nitrogen	0.213		0.113	0.124
Amino nitrogen	0.032		0.027	0.039
Ammonia nitrogen	0.057		0.084	0.049
Reaction	+0.60		−2.10	+4.30
PH	7.0		.....	5.8
Total nitrogen	1.064	15	1.064	1.064
Protein nitrogen	0.762		0.717	0.750
Nonprotein nitrogen	0.302		0.347	0.314
Polypeptid nitrogen	0.213		0.239	0.219
Amino nitrogen	0.032		0.031	0.041
Ammonia nitrogen	0.057		0.078	0.054
Reaction	+0.60		−3.10	+4.70
PH	7.0		8.9	5.8

DISCUSSION

In general, the nitrogenous changes produced by B. coli are qualitatively similar to those found for B. typhosus, B. dysenteriae and B. paratyphosus.[2] Quantitatively, the increase in protein nitrogen, indicative of the growth of the organisms with the transformation of some

of the nonprotein constituents of the medium into the actual bacterial substance, is not very different from that observed in cultures of the pathogenic members of the intestinal group. In other words, the rate of growth of all the microbes studied in this series is relatively about the same. The development in glucose gelatin appears to be more rapid and somewhat more extensive than in the plain gelatin. This is indicated by the slightly greater increase in protein nitrogen in the former medium. There is not much change in the amino nitrogen, but the tendency is toward a reduction in the plain gelatin, whereas the glucose gelatin change in this fraction of the cultural nitrogen is, within the limits of error of the method, practically negligible.

The changes in free ammonia, on the contrary, are unmistakable. In the glucose gelatin, in spite of an apparently more luxuriant growth of the bacteria, the amount of this substance not only fails to increase —on the contrary, there is a slight diminution. This contrasts sharply with the unmistakable increase in the plain, glucose-free gelatin up to the period of maximal development of the culture, namely, the tenth day. At this time, the increase in free ammonia above that of the uninoculated control is very nearly 3%. As this increase is the quantitative measure of the intracellular utilization of protein for energy by the bacteria, the significance becomes apparent. Expressed differently, it may be stated that the increase in protein nitrogen (bacterial protein) in plain gelatin, amounting in terms of nitrogen to 10% of the total nitrogen of the medium, was accomplished at the expense of nearly 3% of nitrogen liberated as free ammonia. In glucose gelatin, on the contrary, approximately 12% of the total nitrogen of the medium was transformed into bacterial substance (protein nitrogen) without the slightest discernible increase in free ammonia. In the former instance (sugar-free gelatin), the requisite energy of the bacteria appears to have been obtained by the combustion of nonprotein nitrogenous constituents, whereas in glucose gelatin the energy was derived in such a manner as to leave the nonprotein constituents relatively intact. This is shown, on the one hand, by the constancy of the amino and ammonia nitrogen fractions, and, on the other hand, by the almost direct parallelism between polypeptid decrease and protein nitrogen increase. This would appear to lend additional weight to the theory that utilizable carbohydrate protects or spares cultural protein from utilization of bacteria for energy.[5] The change in reaction, progressively more

[5] Kendall: Bacteriology, General, Pathological and Intestinal, Ed. 2, 1921, Chapter IV, for literature.

alkaline in the sugar-free medium and progressively more acid in the glucose medium, is indicative of the same phenomenon. It is well known that the products derived from the putrefaction of protein by bacteria are basic, whereas those of the fermentation of carbohydrate are acidic in character and reaction.

It must be admitted that the analytic figures, striking as they are, fail theoretically to eliminate the possibility that the increase in the protein fraction, due to the accumulation of bacteria in the culture, may not be a resultant between an unrecognized breaking down of some of the protein nitrogen of the original medium which is overshadowed and therefore masked by the relatively rapid growth of the bacteria. As the increase in the protein fraction amounts to 10% or more of the total nitrogen of the medium, this possibility clearly exists.

To obtain some evidence on this point, an attempt was made to detect enzymic changes in gelatin which might be caused by the activities of an hitherto undetected proteolytic enzmye produced by B. coli. It must be admitted that all previous evidence is against this view because the organism, by universal consent, is stated not to liquefy gelatin.

The method employed is one familiar to bacteriologists. It consists essentially in adding a small, definite amount of culture to a larger definite amount of 5% gelatin, containing 0.5% phenol to prevent growth. After two or three days' incubation of such cultures of organisms as B. proteus, which excrete soluble enzymes, such sterile mediums will become liquefied without the slightest evidence of growth.[4]

Table 2 shows the results of two experiments, respectively, in which 3 and 6 day cultures of B. coli in plain and glucose gelatin were added to carbol gelatin in the proportions of 5 c c of culture to 95 c c of the carbol gelatin. The results are wholly negative with respect to the demonstration of a soluble proteolytic enzmye. The variations between control and inoculated carbol gelatin mixtures are within the limits of error of the method employed. Under similar conditions, those bacteria which excrete soluble proteolytic enzymes would show physical and chemical signs of activity.

It cannot be claimed of course that this observation absolutely excludes the possibility of the existence of a soluble proteolytic enzyme, but it does make the probability of the occurrence of an enzyme in an active state unlikely. Considering all the factors in the metabolism of

B. coli, as exhibited in tables 1 and 2, it may be stated that the gelatin protein is of little or no value to the organism as a source of nitrogenous energy. The somewhat greater nitrogenous waste attending the growth and activity of B. coli in plain gelatin in comparison with the dysentery and typhoid cultures stands in relation to the fact that

TABLE 2

ENZYME STUDY; CARBOL GELATIN. BACILLUS COLI

	Plain Culture		Glucose Culture	
	Control	Three Days' Incubation	Control	Three Days' Incubation
Total nitrogen	0.675	0.675	0.675	0.675
Protein nitrogen	0.541	0.529	0.541	0.552
Nonprotein nitrogen	0.134	0.146	0.134	0.123
Polypeptid nitrogen	0.096	0.109	0.098	0.087
Amino nitrogen	0.028	0.028	0.028	0.028
Ammonia nitrogen	0.010	0.009	0.008	0.008
Reaction	+0.60	+0.70	+0.90	+0.90
P_H	6.3	6.3	5.9	5.9
Total nitrogen*	0.675	0.675	0.675	0.675
Protein nitrogen	0.552	0.563	0.541	0.541
Nonprotein nitrogen	0.123	0.112	0.134	0.134
Polypeptid nitrogen	0.085	0.084	0.098	0.098
Amino nitrogen	0.028	0.028	0.028	0 028
Ammonia nitrogen	0.010	0.011	0.008	0.008
Reaction	+0.20	+0.20	+1.10	+1.20
P_H	6.6	6.8	5.6	5.6

* 6 days incubation.

progressively pathogenic bacteria produce less deep seated changes, generally speaking, in protein mediums than the parasitic and saprophytic types. The presence of indol in cultures of B. coli and the practical absence of this substance in cultures of B. typhosus, is suggestive but not conclusive evidence on this point.